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Biochemical Analysis of *Lamellidens marginalis* During Nacre Formation

Sakshi S. Dhekane[1] and Nitin A. Kamble[2]
[1-2] Department of Zoology, Shivaji University, Kolhapur, Maharashtra, India
sakshidhekane2024@gmail.com[1]

Abstract: *In the applied sciences, especially in biological world, pearl culture is emerging branch. Now a days freshwater bivalve species Lamellidens marginalis used in pearl formation. Health and bioenergetics are crucial factor in nacre formation so, present work was carried out for comparative analysis of protein, lipid and carbohydrate from interested organs including mantle, gonad, hepatopancreas, gills and foot tissues of freshwater bivalve Lamellidens marginalis. In the process of pearl formation nacre synthesis was continuous process. In our study we dissected experimental animals at 4th 8th and 12th month operated animals were analysed and comparatively subjected for estimation of essential biochemical components glycogen, protein and lipid which serves as an important energy source in the nacre synthesis and secretion. Biochemical results revelled alterations in nutritional components of interested tissues. The obtained results were interpreted for comparative analysis and animal potential for quality pearl synthesis.*

Keywords: carbohydrate, protein, lipid, *Lamellidens marginalis*.

I. INTRODUCTION

The phylum Mollusca under the kingdom animalia is the one of the largest phylum in the metazoan (Parkhaev, 2017), class Bivalvia in the phylum Mollusca holds the record of high number of species, as per available literature these animals were age in exceed of 150 years (Bogan, 2007). The Unionid mollusc *Lamellidens marginalis* has a capability to serves as a good indicator for the freshwater ecosystems (Hameed and Raj,1990), these best bio filters are bountiful in the Indian freshwater habitats. (Jana and Das,1997). Bivalve mollusks are the primary filter-feeders in marine and freshwater systems, making up most of the biomass and exerting control over the structure and function of ecosystem (Dame, 1996 and Strayer et al., 1999). Bioeconomicaly, impact of bivalves on ecosystem processes in freshwater systems is significant. Additionally, they can be important filter feeders and have direct impacts benthic processes occur during their burrowing through sediments. (Vaughn and Hakenkamp,2001) Bivalve's filtration can result in a significant decrease in phytoplankton and other particles in the water column (Kasprzak, 1986; Kryger and RiisgaÊrd, 1988; Welker and Walz, 1998, and Strayer et al., 1999).

The name Kokichi Mikimoto immediately comes to mind when discussing cultured pearls, as he was the first to discover the technique for culturing pearls. These *Lamellidens marginalis* is now used for the production of high-quality image or designer pearls (Siddique et al., 2024). The mantle of the bivalve shows higher metabolic activity (Rosenberg and Hughes 1991). Identification of pearl formation is dependent upon the shell of the bivalve, also the inner body of the bivalve scientifically defines the species for pearl formation. The highly estimated bivalve product is 'pearl', which is a nacreous deposition having a composition of 2-4% of water, 10-14% of organic substance conchiolin and 82-86% of calcium carbonate (aragonite crystals). When any of the foreign particle trapped in between mantle and shell the animal starts to synthesize the protective covering around it and that is nacre.

The majority of living organisms obtain their energy through the metabolic decomposition of carbohydrates. The glycogen acts as a chief reservoir in the tissue, which provides the glucose for the glycogenolysis, as per the physiological demand of the organism, any modification in the environment is recognized to impact the nervous system, which subsequently leads to changes in biochemical processes, particularly those related to carbohydrate

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metabolism (Dongre and Kure, 2014). Bivalves principal metabolic reserve, glycogen, can be used to assess their health and condition; however, using glycogen as a diagnostic tool in aquaculture and biomonitoring were reported comparatively rare (Vodakova and Douda, 2019). The early indication of stress reported as change in physiological conditions, because of increased energy demand associated with stress which often resulted in depletion of glycogen documented as principal storage in carbohydrate in mussels (Naimo et al., 1998).

The high-quality source of animal protein for human consumption are bivalves, because they are reach in protein having > 50% of their dry weight and amino acids > 30g/ 100g of protein (Song et al., 2024). Important factor in the animal tissue composition is protein, the environmental factors as temperature, P^H, food can affect the biochemical content, the influence of temperature is direct as it impacts the metabolic rate of bivalves (Suryawanshi, 2022). Holland (1978), reviewed energy metabolism of different various invertebrate larvae and noticed that neutral lipids, particularly triacylglycerol, were the most significant storage medium utilized during periods of nutritional or environmental stress.

II. MATERIALS AND METHODS

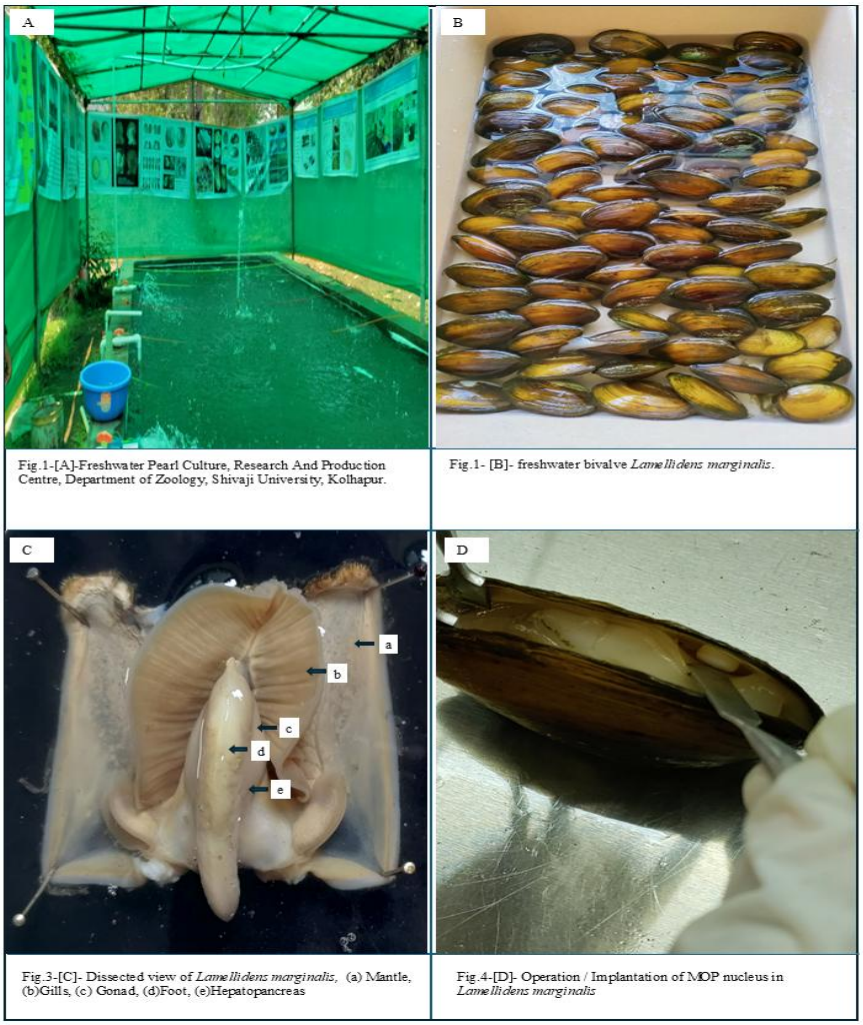

Fig.1-[A]-Freshwater Pearl Culture, Research And Production Centre, Department of Zoology, Shivaji University, Kolhapur.

Fig.1- [B]- freshwater bivalve *Lamellidens marginalis*.

Fig.3-[C]- Dissected view of *Lamellidens marginalis*, (a) Mantle, (b)Gills, (c) Gonad, (d)Foot, (e)Hepatopancreas

Fig.4-[D]- Operation / Implantation of MOP nucleus in *Lamellidens marginalis*

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Collection and selection of animal:

The freshwater bivalves *Lamellidens marginalis* were collected from the Bhogavati River in the Kolhapur district by the hand-picking method and collected in the tubs and cleaned, the animals of 7-8 cm in length, 5-6 cm in breadth and weight of 30-60 gm were selected for the experimental study.

Selection of organs:

Mantle, gonad, hepatopancreas, gills and foot these organs were selected for experimental work. As the mantle and gonad is responsible for nacre synthesis and secretion which is important in pearl culture, hepatopancreas is a main organ for metabolism. The foot is responsible for movement and food capture and gills is the respiratory organ in case of freshwater bivalves *Lamellidens marginalis*.

Experimental design:

The selected animals were maintained in lab for 15 days, after acclimatization 100 animals were operated for mantle cavity implantation of MOP nucleus (half round) and 10 animals were kept unoperated. After completion maintained under the optimum conditions as per the guidelines of CIFA.

Pearl culture:

The selected freshwater bivalves *Lamellidens marginalis* were transferred for preoperative care to laboratory cleaned twice and treated with chloramphenicol (100mg/L) for 24hrs. after 7 days animals were operated without harming the adductor muscle, nucleus of 4-6 mm are grafted in mantle cavity area on both sides. After 4-5 days of post operative care the animals were transferred to main culture tank.

Total 24 animals were differentiated into 4 groups- control, experimental group -I, experimental group -II and experimental group – III and each group contains 6 animals. After successful 4^{th} month, 8^{th} month and 12^{th} month 6 animals were sacrificed from each group. Mantle gonad, hepatopancreas, gills and foot these tissues were removed for biochemical estimations.

Table no. 1. Experimental groups

Sr. No.	Groups	Specificity	No. of animals
1	control	Contains normal-healthy unoperated animals	6
2	experimental group -I	Contains normal-healthy operated animals of 4^{th} month	6
3	experimental group -II	Contains normal-healthy operated animals of 8^{th} month	6
4	experimental group – III	Contains normal-healthy operated animals of 12^{th} month	6

Biochemical procedures:

1. Glycogen estimation by DeZwaan and Zande, 1972.

The animal sacrificed and 100 mg of mantle, gonad, hepatopancreas, foot and gills tissues was removed and homogenised in 1 ml of 30% KOH. The homogenate kept in boiling water bath for 5 min and cooled. 2ml of 96% ethanol were added and kept overnight at 4°C overnight for precipitation. The precipitate was centrifuged at 3000rpm for 15 minutes. After addition of 2ml distilled water in pellet kept at 70°C for 5 minutes. 0.1 ml sample was transferred in triplicates then 0.9

ml distilled water was added in each test tube. Cooled and 5ml of Anthrone reagent was added, then tubes were transferred in boiling water bath for 10 minutes, blank was prepared by adding 1ml H_2SO_4 and 3ml Anthrone reagent. The optical density was observed at 660 nm by using spectrophotometer.

2. Protein estimation by Lowry et al., 1951.

The animal sacrificed and 100mg of mantle, gonad, hepatopancreas, foot and gills tissues were removed and kept for hardening. homogenized in chilled 10ml of 0.8% Nacl and centrifuged at 3000 rpm for 10 min. 0.5 ml supernatant was

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used as a sample in triplicates ,5 ml Lowry's C reagent was added and kept for 10 min at room temperature. Then 0.5 ml of Folin phenol reagent was added and kept at room temperature for 30 minutes. The optical density was observed at 660 nm by using spectrophotometer. Blank was prepared similarly only without the addition of sample, instead of sample 0.5 ml of distilled water was added.

3. Total lipid content by Barnes and Blackstock, 1973.

The animal sacrificed and 100 mg tissue was removed and kept for hardening for 1-2 hrs. homogenate was prepared in 10 ml of Floch's mixture and filtered. From filtrate 1ml of sample was taken kept in boiling water bath for 40°C for 2 hrs. 1ml of concentrated H_2SO_4 was added and kept in boiling water for 10 minutes. After cooling 0.2 ml of sample in triplicates, 2ml of vanillin reagent was added, pink colour developed. The optical density was measured by spectrophotometer at 540 nm. The blank was prepared by adding 0.2 ml distilled water and 2ml of vanillin reagent.

III . RESULT

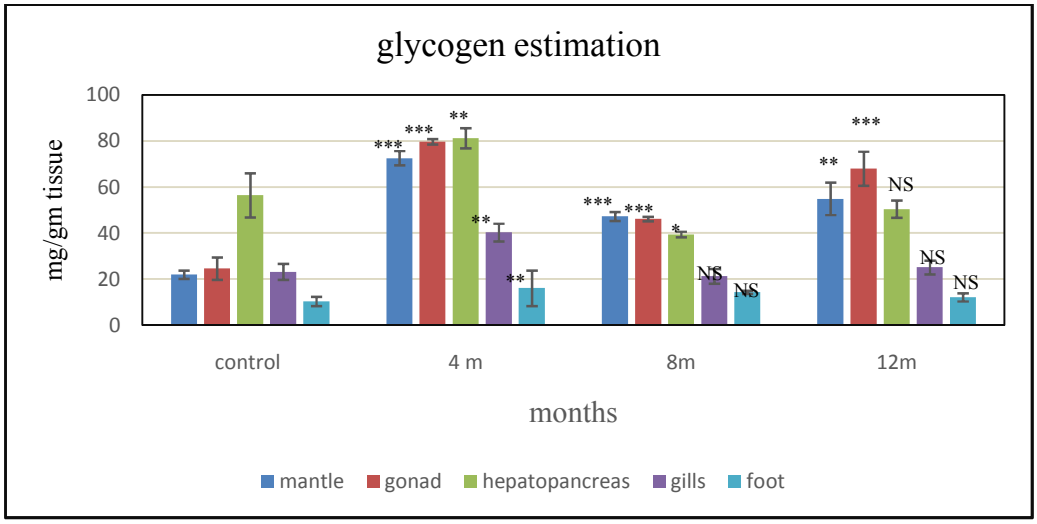
The present research work was carried out on some tissues of *Lamellidens marginalis* such as mantle, gonad, hepatopancreas, gills and foot. Total 24 animals were differentiated into 4 groups, control, experimental group -I, experimental group -II and experimental group – III as mentioned in table no.1. each group contains 6 animals respectively.

Sr. No.	Tissues	control	experimental group -I	experimental group -II	experimental group – III
1.	Mantle	21.97 ±1.78	72.54 ±3.10***	47.21± 1.95***	54.90 ± 7.01**
2.	Gonad	24.57 ±4.85	79.68 ±1.20***	46.16 ± 1**	67.96 ± 7.24***
3.	Hepatopancreas	56.42 ±9.53	83.19 ± 4.34**	39.42 ± 1.24*	50.42 ± 3.73^{NS}
4.	Gills	23.21 ±3.50	40.30 ±3.83**	21.3 ± 3.19^{NS}	25.10 ± 3.01^{NS}
5.	Foot	10.38 ±2.04	16.07 ± 7.73^{NS}	14.34 ± 0.7*	12.14 ± 1.77^{NS}

Table no.2. glycogen contains from control and experimental groups from tissues mantle, gonad, hepatopancreas, gills and foot in mg/100 mg tissue

P-value:

P< 0.001 Highly significant***, P< 0.01 Statistically significant**, P<0.05Almost significant*, P> 0.05 non-significant



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Graph no. 1: showing glycogen concentration (mg/g tissue) from experimental groups control, experimental group -I, experimental group -II and experimental group–III, from tissues mantle, gonad, hepatopancreas, gills and foot.

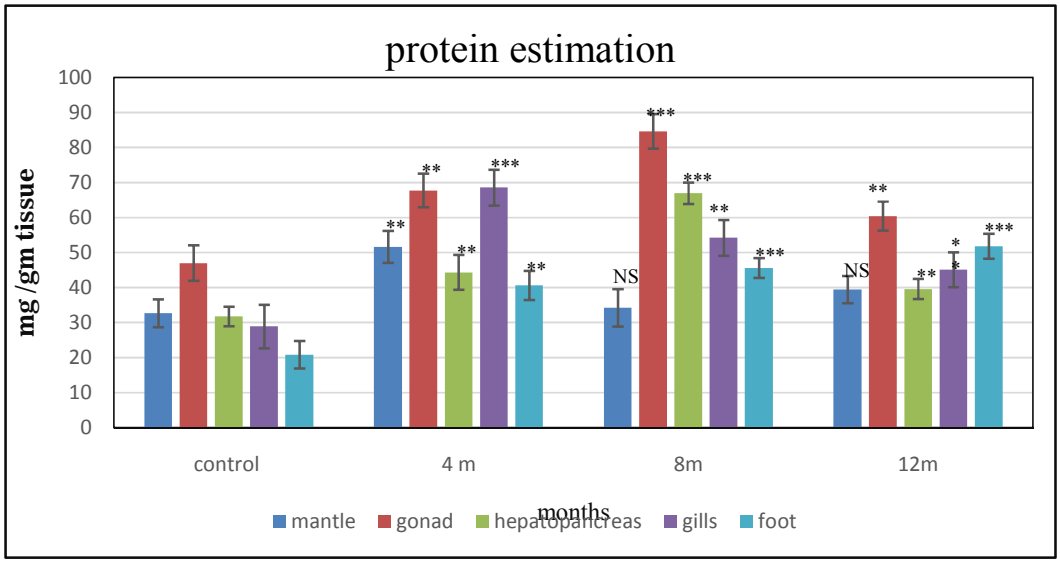
In control group, the hepatopancreas shows the higher glycogen level as compared to other tissues, whereas gills showed the least glycogen. In experimental group -I, II and III a marked elevation in glycogen content was observed from mantle and gonad in all experimental groups i.e. highly significant ,mantle (Ex.g-I- 72.54 ± 3.10***, Ex.g-II – 47.21 ± 1.95*** Ex.g-II – 54.90 ± 7.01**) and in gonad (Ex.g-I – 79.68 ± 1.20 ***, Ex.g-II – 46.16 ± 1**, Ex.g-II – 67.96 ± 7.24***). The hepatopancreas shows statistically significant increase in experimental group -I 83.19 ± 4.34** and almost significant in experimental group -II (39.42 ± 1.24*), while in experimental group -III nonsignificant increase (50.42 ± 3.73^{NS}) was observed as compared with control. Gills show moderate but significant increase in experimental group- I (40.30 ±3.83) while in experimental group II 21.3 ± 3.19and III 25.10 ± 3.01it is non-significant. Foot shows nonsignificant rise as compared to control in experimental group I – 16.07 ± 7.73 and III 14.34 ± 0.73 although in experimental group II it shows almost significant rise.

Table no.3. protein contains from control and experimental groups from tissues mantle, gonad, hepatopancreas, gills and foot in µg/100 mg tissue.

Tissues	control	experimental group -I	experimental group -II	experimental group – III
Mantle	32.68 ±3.98	51.66±4.57**	34.25± 5.35NS	39.41 ±3.87NS
Gonad	47 ± 5.06	67.74 ± 4.78**	84.63 ± 4.92***	60.44 ± 4.12**
Hepatopancreas	31.77 ± 2.79	44.36 ± 4.95**	66.96 ± 3.05***	39.59 ± 2.88**
Gills	28.89 ± 6.20	68.55 ± 5.18***	54.20 ± 5.17**	45.1 ± 4.96**
Foot	20.85 ± 3.92	40.62 ± 4.17**	45.58 ± 2.82***	51.84 ± 3.57 ***

P-value:

P< 0.001 Highly significant***, P< 0.01 Statistically significant**, P<0.05Almost significant*, P> 0.05 non-significant



Graph no.2. showing protein concentration (µg/100mg tissue) from experimental groups control, experimental group -I, experimental group -II and experimental group – III from tissues mantle, gonad, hepatopancreas, gills and foot.

The protein estimation from the mantle, gonad, hepatopancreas, gills and foot from experimental group I, II and III showed increase in protein contain as compared to control group i.e. showed in graph no.1. the mantle tissue shows an increase in protein levels, in experimental group I this increase is 51.66 ± 4.57 statistically significant (p-value <0.01),

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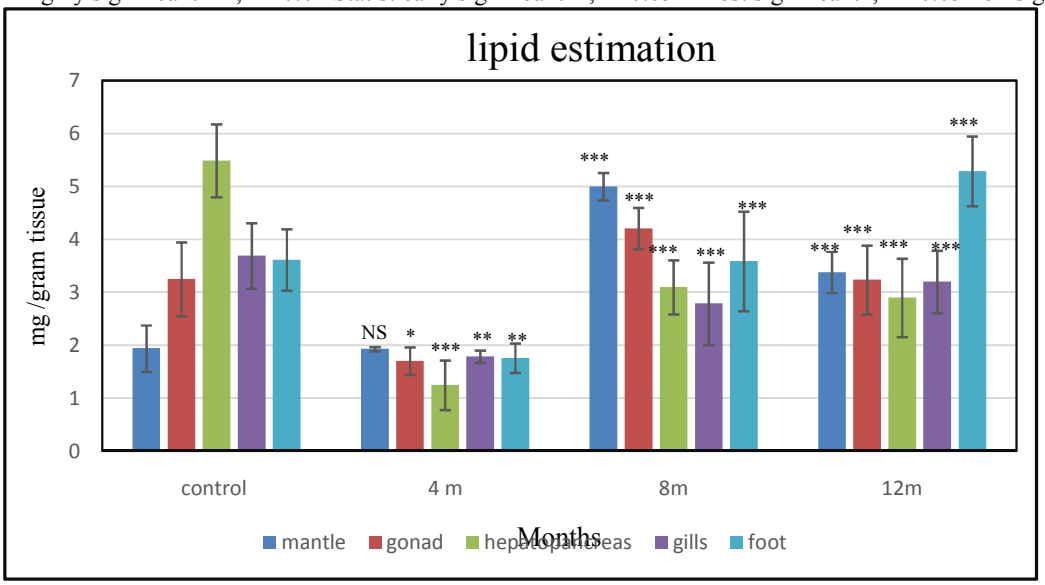
whereas in experimental group II 34.25 ± 5.35 and experimental group III 39.41 ± 3.87 non-significant (p-value > 0.05) increase was observed compared with control. In gonad rise was significant in experimental group I 67.74 ± 4.78 and experimental group III 60.44 ± 4.12; highly significant (p-value <0.001) in experimental group II 84.63 ± 4.92. The hepatopancreas shows significant increase in experimental group I 44.36 ± 4.95 and experimental group III 39.59 ± 2.88 whereas, in experimental group II highly significant 66.96 ± 3.05. The gills show statistically significant protein elevation in experimental group II 54.20 ± 5.17 and experimental group III 45.1 ± 4.96 and highly significant increase from experimental group I 68.55 ± 5.18. The foot indicates protein mount up was significant in experimental group I 40.62 ± 4.17 and this mount up in experimental group II and III was highly significant.

Table no. 3. Lipid contains from control and experimental groups from tissues mantle, gonad, hepatopancreas, gills and foot in µg/100 mg tissue

Sr. No.	Tissues	control	experimental group -I	experimental group -II	experimental group – III
1.	Mantle	1.94± 0.44	1.93±0.04 ^{NS}	5 ±0.26***	3.38 ± 0.39**
2.	Gonad	3.58± 1.1	1.70±0.61*	4.21 ± 0.39^{NS}	3.24 ± 0.65^{NS}
3.	Hepatopancreas	5.49± 0.69	1.25± 0.47***	3.10 ± 0.51**	2.90 ± 0.74**
4.	Gills	3.69 ± 0.62	1.78± 0.12**	2.79 ± 0.78**	3.20 ± 0.59^{NS}
5.	Foot	3.61 ± 0.58	1.76± 0.28 **	3.59 ± 0.94^{NS}	5.29 ± 0.66 **

P-value:

P< 0.001 Highly significant***, P< 0.01 Statistically significant**, P<0.05Almost significant*, P> 0.05 non-significant



Graph no. 3: showing lipid concentration (µg/100mg tissue) from experimental groups control, experimental group -I, experimental group -II and experimental group–III, from tissues mantle, gonad, hepatopancreas, gills and foot.

In control group, hepatopancreas showed relatively higher lipid content indicating stored lipid is naturally abundant in the tissue whereas the mantle shows comparatively lower lipid levels.

In experimental group I, lipid level decreased as compared to other groups. this decrease was non-significant in mantle 1.93 ± 0.44 while almost significant in gonad 1.70 ± 0.61, highly significant in hepatopancreas 1.25 ± 0.47, significant in gills 1.78 ± 0.12 and foot 1.76 ± 0.28.

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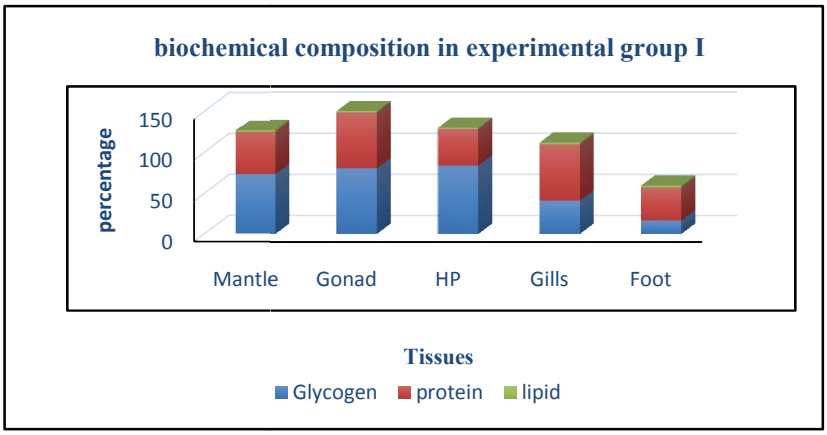
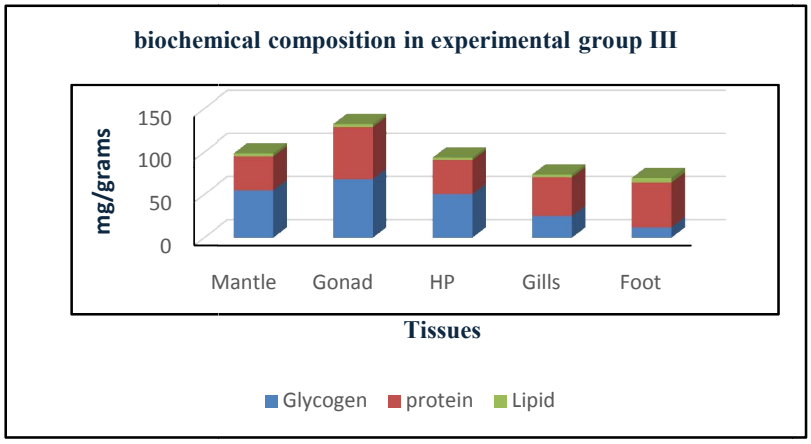
In experimental group II, the lipid contain was increased highly significantly in mantle 5 ± 0.26, significant in hepatopancreas 3.10 ± 0.51 and gills 2.79 ± 0.78 and non-significantly in gonad and foot. Whereas in experimental group III, the lipid contain was increased significantly in mantle, hepatopancreas and foot and non-significantly in gonad and gills.



Graph no. 4: showing glycogen, protein and lipid contain in experimental group -I from tissues mantle, gonad, hepatopancreas, gills and foot.

Graph no. 5: showing glycogen, protein and lipid contain in experimental group -II from tissues mantle, gonad, hepatopancreas, gills and foot.



Graph no. 6: showing glycogen, protein and lipid contain in experimental group -III from tissues mantle, gonad, hepatopancreas, gills and foot.

Tissue	Glycogen	protein	Lipid
Mantle	47.21	34.25	5
Gonad	46.16	84.63	4.21
HP	39.42	66.96	3.1
Gills	21.3	54.2	2.79
Foot	14.34	45.58	3.59

Table No. 5: showing glycogen, protein and lipid contain in experimental group -I from tissues mantle, gonad, hepatopancreas, gills and foot.

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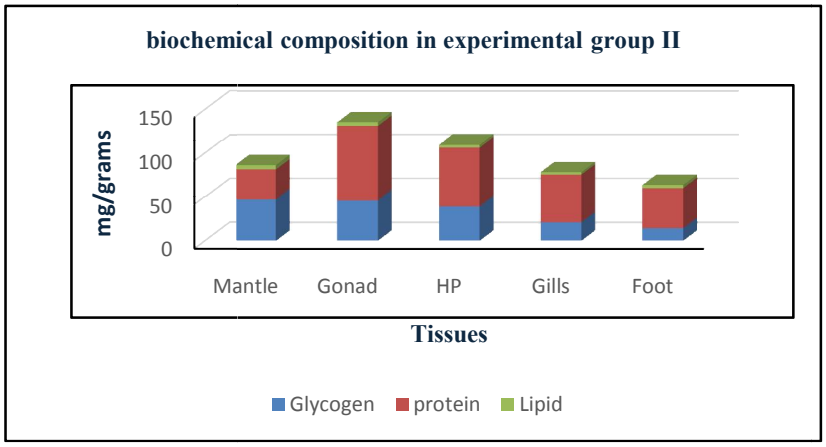

Table No. 6: showing glycogen, protein and lipid contain in

Tissue	Glycogen	protein	lipid
Mantle	72.54	51.66	1.93
Gonad	79.68	67.74	1.7
HP	83.19	44.36	1.25
Gills	40.3	68.55	1.78
Foot	16.07	40.62	1.76

experimental group -II from tissues mantle, gonad, hepatopancreas, gills and foot.

Table No. 7: showing glycogen, protein and lipid contain in experimental group -III from tissues mantle, gonad, hepatopancreas, gills and foot.

Tissue	Glycogen	protein	Lipid
Mantle	54.9	39.41	3.38
Gonad	67.96	60.44	3.24
HP	50.42	39.59	2.9
Gills	25.1	45.1	3.2
Foot	12.14	51.84	5.29

The glycogen, protein and lipid are the major biochemical constituents in the bivalves. Each tissues shows the different concentration of macromolecules mentioned in table no. 5, 6, 7 and graph no.4, 5, 6. In experimental group I, the hepatopancreas showed highest glycogen whereas lowest was observed in foot. On the other hand, the major protein and lipid was observed in foot. In experimental group II, mantle showed highest glycogen and foot showed high amount of protein and lipid. The similar observations regarding protein and lipid were observed from foot and highest glycogen was observed in mantle tissue.

These experimental results indicate the highest glycogen level was from hepatopancreas in control group and in experimental group I i.e. in the 4^{th} month of the pearl formation whereas in experimental group II (8^{th}) and III (12^{th}) month the mantle tissue exhibited highest glycogen levels, suggesting that the glycogen plays a crucial role in providing energy for synthesis and secretion of nacreous layer during pearl formation.

IV. DISCUSSION

The present research work examines that in control condition highest glycogen, protein and lipid was observed in hepatopancreas, as it is an important tissue in metabolic operations. Although lowest glycogen and protein was seen in gills, the gills is not a muscular tissue, on the other hand mantle shows lowest stored lipids.

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In experimental groups I, II and III the protein was significantly increased in all tissues as compared with control group. protein is the primary structural and functional component which was used for growth, repair, metabolism or enzymatic functions in bivalves (Lopez-Pedrouso et al., 2022), therefore it's possible that the impact of implantation enhanced the protein for development of nacre or pearl. The maximum protein contain were reported from hepatopancreas and minimum was from foot by (Suryawanshi and Kulkarni, 2019).

The glycogen was found to be statistically increased from mantle, gonad and hepatopancreas, the lowest glycogen was observed in gills and foot. glycogen is main short term carbohydrate store in bivalves and is quickly utilized to provide energy during stress, hypoxia or starvation (Vodakova & Douda, 2019). The reserve glycogen storage was found highest in mantle and gonad because these tissues are responsible for pearl formation of pearl and in hepatopancreas as it is a metabolic reservoir. Suryawanshi and Kulkarni, 2019 reported highest glycogen in mantle, gonad and hepatopancreas tissues except gills which showed minimum glycogen. (Balamurugan and Chakravarthy, 2024), documented evidence of seasonal variation in biochemical composition of subcellular fractions in tissues of *Lamellidens marginalis* discovered highest glycogen, protein and lipid from digestive gland.

Lipids are long-term reserves of energy, crucial for membrane integrity and are particularly important for gametogenesis in reproduction (Balamurugan, 2021). The lipid content was higher in experimental group II and III than in the control and experimental group I. this increase may indicate that the implanted nucleus in the bivalve body is associated with higher energy demand during the process of implantation. At the same time, synthesis and secretion of nacre were enhanced, and as secretory activity increased, the stored energy in the tissues was utilized during the first four months. Because lipids were being extensively used as an energy source, the animals appear to have stored additional energy in the form of lipids for subsequent utilization. (Jagtap, 2021) concluded depletion of lipid in gonad, gills and digestive gland from *Lamellidens marginalis* due to increased period of acute concentration of tributyltin chloride due to stress condition. (Shafakatullah et.al.,2014), concluded fluctuations in macronutrient proteins, lipids and carbohydrate in *parreysia spp*. of Periyar river, Kerala, India throughout the seasons, protein and lipid levels showed seasonal accumulation during pre and post monsoon periods, followed by decline in monsoon and winter, whereas carbohydrate reserves decreased in pre and post monsoon but increased during monsoon and winter. (Lagade et.al., 2015), concluded physiological activity, nutritive value and meat quality of Soletellina diphos exhibit high seasonal variation.

V. CONCLUSION

The present investigation enlight's regarding efficiency of organism for energy used during nacre formation. Experimental analysis of biochemical components of animal showed that basic energy is needed for nacre formation. Organ wise energy utilization was found were after 12 months of period. Overall gonadal part showed heavy fluctuations as compared to other organization. Hepatopancreas showed accelerated rate of metabolism provoking organizational bioenergetics. Animals own capacity during nacre formation enhanced during the period of experiment. It indicated the positive response of animal towards operative measures carried out for purpose of pearl formation. *Lamellidens marginalis* as an experimental animal found to be efficient organism for freshwater pearl culture.

Conflict of interest:

The others have no any conflict of interest

Acknowledgement:

Authors are thankful to the Department of Zoology, Shivaji University, Kolhapur for providing a facility to perform the research work.

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